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APE1, the DNA base excision repair protein, regulates the removal of platinum adducts in sensory neuronal cultures by NER

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ABSTRACT

Peripheral neuropathy is one of the major side effects of treatment with the anticancer drug, cisplatin. One proposed mechanism for this neurotoxicity is the formation of platinum adducts in sensory neurons that could contribute to DNA damage. Although this damage is largely repaired by nuclear excision repair (NER), our previous findings suggest that augmenting the base excision repair pathway (BER) by over-expressing the repair protein APE1 protects sensory neurons from cisplatin-induced neurotoxicity. The question remains whether APE1 contributes to the ability of the NER pathway to repair platinum-damage in neuronal cells. To examine this, we manipulated APE1 expression in sensory neuronal cultures and measured Pt-removal after exposure to cisplatin. When neuronal cultures were treated with increasing concentrations of cisplatin for two or three hours, there was a concentration-dependent increase in Pt-damage that peaked at four hours and returned to near baseline levels after 24 h. In cultures where APE1 expression was reduced by ~80% using siRNA directed at APE1, there was a significant inhibition of Pt-removal over eight hours which was reversed by overexpressing APE1 using a lentiviral construct for human wtAPE1. Overexpressing a mutant APE1 (C65 APE1), which only has DNA repair activity, but not its other significant redox-signaling function, mimicked the effects of wtAPE1. Overexpressing DNA repair activity mutant APE1 (226 + 177APE1), with only redox activity was ineffective suggesting it is the DNA repair function of APE1 and not its redox-signaling, that restores the Pt-damage removal. Together, these data provide the first evidence that a critical BER enzyme, APE1, helps regulate the NER pathway in the repair of cisplatin damage in sensory neurons.

1. Introduction

It is well established that cisplatin, which is a primary therapy in a number of cancers [1–5] cause peripheral neuropathy in a significant number of patents [1,6–8]. The onset and severity of the neuropathy correlate with the cumulative dosage of the drug administered and with the duration of therapy [1,9]. The severity of the neuropathy can warrant stopping therapy and in many cases, the toxicity is maintained after therapy is discontinued [8,10,11]. To date, there are no therapies available to prevent or reduce cisplatin-induced peripheral neuropathy (CIPN) although a number of agents have been tried [12].

The cellular mechanisms mediating cisplatin-induced neuropathy remain to be determined, but evidence suggests that the neurotoxicity is secondary to a direct action of this drug on the cell bodies of sensory neurons. In patients and in animals receiving cisplatin, the highest concentrations of the drug and of platinum adducts are observed in the cell bodies of sensory neurons and in peripheral sensory neurons [6,13]. Furthermore, cisplatin caused the formation of intrastrand and interstrand Pt-adducts in rat sensory neurons and the level of these adducts raises with increasing the cumulative dose of cisplatin [13,14]. The levels of platinum adducts also correlate directly with the ability of cisplatin to cause histopathology, axonal degeneration and/or changes in nerve conduction [14,15].

Since nuclear excision repair (NER) is the major pathway for removing platinum adducts [16], alterations in the pathway should influence cisplatin-induced neuropathy. Indeed, in mice deficient in Xeroderma pigmentosum complementation group A (XPA) or group C (XPC), complexes necessary for NER, there is an increase

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in platinum adduct formation in sensory neurons and in satellite cells of the dorsal root ganglia compared to wild type mice [17]. Furthermore, in XPC deficient mice, removal of adducts is reduced over time and mice with compromised NER show an increase in symptoms of neuropathy after cisplatin treatment compared with mice with intact NER [17]. These data suggest that the NER pathway may be critical for reducing cisplatin-induced neuropathy.

In previous work from our laboratory, however, we demonstrated that augmenting the base excision repair (BER) pathway by increasing the expression of apurinic/apyrimidinic endonuclease (APE1) is neuroprotective against cisplatin-induced toxicity in isolated sensory neurons [18]. We and others also have shown that cisplatin increases production of reactive oxygen species (ROS) and that oxidative stress may contribute to cisplatin-induced toxicity [18–21]. Since oxidative DNA damage is repaired by BER pathway [7,21–25], it is interesting to speculate that this pathway contributes to DNA repair after sensory neurons are exposed to cisplatin. The question remains, whether augmenting the BER pathway affects the ability of the NER pathway to remove platinum adducts from sensory neurons.

To address this question, we used a DNA slot blot method to quantitatively measure platinum adducts after exposure to cisplatin in sensory neuronal cultures with a reduction or overexpression of APE1. We found that reducing expression of APE1 inhibits the repair of cisplatin adducts damage, while adding back APE1 with repair activity, but not the redox-signaling function restores repair of cisplatin damage. Additionally, two proteins involved in early steps of NER, RPA and XPA were affected when APE1 levels were altered. These data support an interaction between the BER and NER pathway that promotes removal of platinum adducts in sensory neurons.

2. Materials and methods

2.1. Materials

Tissue culture supplies were obtained from Invitrogen (Carlsbad, CA), and routine chemicals including cisplatin from Sigma Chemical Company (St. Louis, MO). Normocin was purchased from InvivoGen (San Diego, CA) and nerve growth factor from Harlan Bioproducts for Science, Inc. (Indianapolis, IN). Optiprep was obtained from NYCOMED PHARMA AS (Oslo, Norway). The transfecting reagent, Neuroporter® was purchased from Gene Therapy Systems (San Diego, CA). QIAamp DNA Mini kit was purchase from Qiagen (Valencia, CA), and Western blotting detection ECL system from Amersham GE Healthcare (Pittsburgh, PA). Mouse monoclonal anti-human APE1 antibodies were produced in the Kelley laboratory and are available from Novus Biologicals, Littleton, CO. An anti-1,2-Pt-(GpG) monoclonal antibody was purchased from Oncolyze (Essen, Germany), peroxidase-coupled secondary antibody from Sigma Chemical Company (St. Louis, MO), goat anti-mouse HRP conjugated IgG secondary antibody from Zymed Laboratories Inc. (San Francisco, CA), actin antibodies from Thermo (Fremont, CA), and HA rat monoclonal antibodies from Roche Applied Science (Mannhiem, Germany). The rabbit polyclonal RPA70 (70 kDa subunit of replication protein A, RPA) antibody was from Novus Biologicals (Littleton, CO), while the rabbit polyclonal XPA antibody was from Santa Cruz Biotechnology (Dallas, TX). The Animal Care and Use Committee at Indiana University School of Medicine, Indianapolis, IN approved all procedures used in these studies.

2.2. Cell culture

Sensory neuronal cultures were prepared for the dorsal root ganglia (DRG) taken from male Sprague–Dawley rats (150–175 g) as previously described [26]. Briefly, rats were euthanized by CO_2 asphyxiation and the DRG removed from the length of the spinal column. The DRG were incubated in collagenase, cells mechanically dissociated, and ~60,000 cells were plated into each well of poly-D-lysine and laminin-coated six-well culture plates. Cells were maintained in F-12 media (Invitrogen) supplemented with 10% horse serum, 2 mmol/L glutamine, 100 μg/mL normocin, 50 μg/mL penicillin, 50 μg/mL streptomycin, 50 μmol/L 5-fluoro-2′-deoxyuridine (Invitrogen), 150 μmol/L uridine, and 30 ng/mL of nerve growth factor (Harlan Bioproducts for Science, Inc.) in 3% CO_2 at 37 °C. Growth medium was changed every other day.

2.3. Transfection with siRNAs

To reduce APE1 expression in sensory neuronal cultures, on day 3 in culture, cells were exposed to 100 nM APE1-siRNA or a scrambled control (SCsiRNA) for 48 h as previously described [27,28]. Briefly, the growth media was replaced with 0.5 mL of Optimem I medium containing 10 μL of the transfecting reagent Neuroporter in the absence or presence of the 21-mer oligonucleotide double-stranded small interfering RNA (siRNA) to rat APE1 (5′-GUCUGGUAAGACUGGAGUACC-3′) or a scramble siRNA (5′-CCAUGAGGUCAGCAUGGUCUG-3′). Fresh medium (0.5 mL) without antibiotics was added after 24 h of incubation, and after an additional 24 h, the medium was replaced with normal medium containing antibiotics and cell growth maintained.

2.4. Lentivirus infection

Lentiviral constructs were produced using the Gateway® LR Clonase™ II (Invitrogen Life Technologies) enzyme mix to move wtAPE1-IRES-EGFP, C65APE1-IRES-EGFP, or 266 + 177APE1-IRES-EGFP into the lentiviral transfer vector, pCSCGW which we obtained from the Vector Production Facility (Indiana University School of Medicine). In all instances, constructs were derived from human APE1 and had a hemagglutinin epitope (HA) tag on the amino end of the molecule and expressed using the cyclomegalovirus promoter. We used human APE1 since it has a base pair difference for rat APE1 and thus its expression is not inhibited by rat siRNA [29]. The constructs were transformed into STBL3 *Escherichia coli* (Invitrogen Life Technologies) and selected using zeocin resistance and ccdB^s sensitivity. The plasmids were isolated using the EndoFree Plasmid Maxi Kit (IBI Scientific, Peosta, IA) and PCR used to confirm that the destination vector contained the appropriate APE1 insert.

On day one of viral production, T175 flasks were plated with 1.2×10^7 293FT cells in 25 mL medium and grown to 80–90% confluence at 37 °C in 5% CO_2 for 24 h. On day 2, the transfer vector, pCSCGW with a gene-of-interest insert, in combination with a 3rd generation packaging combo: pMDG-VSVG, pMDLg, pRRE-gag/pol, and pRSV-Rev were transfected into 293FT cells using the Profection Mammalian Transfection System (Promega). The four plasmids, transfer, envelope, packaging, and expression ratios (μg) were 30.4:10.4:15.2:7.6 of each plasmid for a T175 flask. On day 3, the media was changed and on day 4–5 medium was collected and the viral containing supernatant filtered using a 0.45 μM SFCA syringe or flask filter. The virus was concentrated using Centricon Plus-70 Centrifugal Filters (Millipore), collected, aliquoted, and stored at −80 °C and the viral titers were determined using QuickTiter™ Lentivirus Quantitation Kit (Cell Biolabs, Inc.) For infection, cells were maintained in culture and 20 μl of the concentrated virus added to the cultures on day 5. The virus was removed on day 7 and the cells maintained in culture for an additional 5 days in regular media.